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PATENT
Attorney Docket No. 016777/0311

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF APPEALS AND PATENT INTERFERENCES

In re Application of:

Gautvik *et al.*

Serial No.: 08/340,664

Filed: November 16, 1994

For: PRODUCTION OF HUMAN PARA-
THYROID HORMONE FROM
MICROORGANISMS



Group Art Unit: 1646

Examiner: L. Spector

#37
M.G.J.
5/31/99

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

BRIEF ON APPEAL

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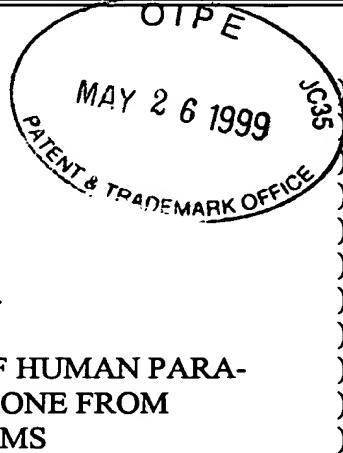
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BRIEF ON APPEAL
UNDER 37 C.F.R. § 1.191

This Appeal Brief is being filed in triplicate together with a check in the amount of \$300.00 covering the appeal fee. If this fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to deposit account 19-0741.

This is an appeal from the Office Action dated June 6, 1998, and the Advisory Action dated January 7, 1999, finally rejecting claims 31-35 under 35 U.S.C. § 112, second paragraph, 35 U.S.C. § 102(b), and 35 U.S.C. § 103.

REAL PARTY IN INTEREST

The real party in interest in this appeal is the assignee, Allelix Biopharmaceuticals, Inc. by virtue of an assignment executed by Astra Aktiebolag, a copy of which is attached as APPENDIX 1.

RELATED APPEALS AND INTERFERENCE'S

Neither appellant, appellant's legal representative, nor assignee are aware of any related appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

STATUS OF CLAIMS

Claims 31-35, 6-10, 12, 14, and 16-20 pending in the application. Claims 6-10, 12, 14, and 16-20 have been withdrawn from consideration and claims 31-35 have been finally rejected. Claims 31-35, set forth in APPENDIX 2, are on appeal

STATUS OF AMENDMENTS

The Advisory Action dated January 7, 1999, indicates that the Amendment submitted on October 26, 1998 in response to the final Office Action will not be entered on appeal. Accordingly, the claims set forth in APPENDIX 2 do not include this amendment.

The Advisory Action also stated that the Terminal Disclaimer for U.S. Patent No. 5,010,010 has been recorded. Thus, the obviousness-type double patenting rejection of claims 31-35 over claims 1 and 21-30 of commonly-assigned U.S. Patent No. 5,010,010, stated in the Office Action of June 3, 1998, is moot.

An amendment to the claims is being filed concurrently herewith in accordance with MPEP § 1207. This amendment clarifies the subject matter of claims 31-35,

adding grammatical corrections to the claims, and cancels non-elected claims 6-10, 12, 14, and 16-20. The claims set forth in APPENDIX 3 include these amendments.

SUMMARY OF INVENTION

The present invention is directed to recombinant human parathyroid hormone (hPTH). hPTH is an important regulator of calcium metabolism in mammals and is also related to several mammalian diseases, such as milk fever, acute hypocalcemia, and otherwise pathologically altered blood calcium levels. *See* page 2, lines 15-19, of the Application. Through its action on target cells in bone and kidney tubuli, hPTH increases serum calcium and decreases serum phosphate, while opposite effects are found regarding urinary excretion of calcium and phosphate. *See* page 5, lines 27-31, of the Application. hPTH is useful, for example, as a component of a diagnostic kit or as a therapeutic in human and veterinary medicine. *See* page 2, lines 20-22, of the Application.

Prior to the present invention, hPTH was commercially available only in very small quantities at high cost, partly because synthesis of the compound was difficult and complex. *See* page 1, lines 33-38, of the Application. In addition, such commercially available compositions were impure, and contained degradation products, isomers, or fragments of hPTH. Recombinant production of hPTH was hampered by the discovery that *E. coli* degrades human hPTH.

Applicants have overcome the problems of the prior art and discovered substantially homogenous hPTH proteins that can be made in high yield using microorganisms, such as *E. coli* and yeast. Stable production of intact hPTH by a microorganism, such as *E. coli* or yeast, was not described prior to the present invention. *See* page 3, lines 3-8; and page 5, lines 1-6, of the Application. Moreover, the hPTH of the invention is superior to prior art hPTH compositions in that it is more than 95% pure. *See* page 5, lines 19-22, of the Application. Biological activity experimental results support the purity of Applicants' recombinant hPTH. As shown in Fig. 2 and explained on page 1264 of Olstad et al., "Differences in Binding Affinities of Human PTH(1-84) do not Alter Potency: A Comparison Between Chemically Synthesized Hormone, Natural and Mutant Forms,"

Peptides, 15:1261-1265 (1994) (Exhibit F to the Declaration of Dr. Gautvik, APPENDIX 5), the recombinant hPTH of the present invention has significantly higher biological activity compared to material produced by solid phase chemical synthesis. The recombinant protein exhibited an almost **four fold increase** in its ability to stimulate intracellular cAMP accumulation as compared to synthetically produced hPTH, i.e., the EC₅₀ values for synthetic and recombinant protein were 1.5 nM and 5.7 nM, respectively. (Declaration of John E. Maggio, dated March 5, 1996, originally submitted with the Amendment filed on March 7, 1996, at ¶ 25 (APPENDIX 4); Declaration of Kaare M. Gautvik, dated February 29, 1996, and submitted with the Amendment filed on March 7, 1996, at ¶ 12 (APPENDIX 5).) Moreover, the recombinant hPTH of the invention achieved a higher maximal response or efficacy when compared to synthetic material. Maggio Decl. at ¶ 26; Gautvik Decl. at ¶ 12. This means that no amount of synthetic material could provide the same efficacy as a maximal dose of recombinant hPTH. Maggio Decl. at ¶ 26; Gautvik Decl. at ¶ 12. Applicants' invention satisfies a long-felt need in the art for substantially homogeneous hPTH(1-84) protein.

ISSUES

- I. Whether claims 33-35 are patentable under 35 U.S.C. § 112, second paragraph.
- II. Whether claims 31 and 32 are patentable under 35 U.S.C. § 102(b) or, in the alternative, under 35 U.S.C. § 103, over Brewer et al. (U.S. Patent No. 3,886,132).
- III. Whether claims 31-34 are patentable under 35 U.S.C. § 103 over Breyel et al. ("Synthesis of Mature Human Parathyroid Hormone in *Escherichia coli*," 3rd Eur. Cong. Biotechnol., 3:363-369 (1984)), or Sung et al. ("Hybrid Gene Synthesis: Its Application to the Assembly of DNA Sequences Encoding the Human Parathyroid Hormones and

Analogues," *Biochem. Cell. Biol.*, 64:133-138 (1986)), or Mayer et al. (EP 0 139 076), or any reference of the three in view of Kaisha et al. (GB 2 092 596).

GROUPING OF CLAIMS

The claims do not stand or fall together, but instead the following groups are separately patentable:

Group I: Claims 31 and 32

Group II: Claims 33-35

Group I is separately patentable from Group II, as indicated by the Examiner, because the claims of Group II require that the claimed protein be produced by a recited recombinant method. In contrast, the claims of Group I do not recite such limitations. As a result, only the claims of Group II were rejected by the Examiner under 35 U.S.C. § 112, second paragraph, for allegedly failing to recite a required element of the transformation process.

ARGUMENT

I. CLAIMS 33-35 ARE PATENTABLE UNDER 35 U.S.C. § 112, SECOND PARAGRAPH BECAUSE A SECRETORY LEADER SEQUENCE IS NOT AN ESSENTIAL ELEMENT OF THE CLAIMED INVENTION AND, THEREFORE, THE CLAIMS NEED NOT RECITE SUCH AN ELEMENT TO BE DEFINITE

Claims 33-35 were rejected under 35 U.S.C. § 112, second paragraph, as being allegedly incomplete for omitting essential elements, such as a secretory leader sequence. Office Action at pages 2-3. Applicants respectfully traverse this ground for rejection.

Claims 33-35 recite a substantially homogeneous recombinant human parathyroid hormone (hPTH (1-84)) protein prepared by (i) providing a microorganism containing exogenous DNA encoding hPTH (1-84); (ii) culturing the microorganism to allow expression of the exogenous DNA, thereby producing hPTH (1-84); and (iii) purifying the hPTH (1-84) as a substantially homogeneous protein. Claims 34 and 35 recite the protein of claim 33, wherein the microorganism is *E. coli* and yeast, respectively.

The use of a particular leader sequence is not "the subject matter applicants regard as their invention." 35 U.S.C. § 112, second paragraph. Rather, the invention is directed to an intact and essentially homogeneous hPTH(1-84) protein. Applicants teach that a variety of leader sequences can be employed in the recombinant production of hPTH according to the invention. See page 5, lines 6-10, of the Application. This is significant because claim language must be read in light of the specification as it would be interpreted by one of ordinary skill in the art. *In re Goodwin*, 198 U.S.P.Q. 1 (CCPA 1978); *In re Moore*, 169 U.S.P.Q. 236, 238 (CCPA 1971). *But claim requires no leader*

Moreover, the use of microbiological techniques employing recombinant DNA to produce species-different peptides was known prior to the invention. See page 2, lines 5-14, of the Application. In addition, leader sequences for recombinant production of proteins was well known in the art prior to Applicants' earliest claimed priority date of October 22, 1996. For example, Watson et al., "Recombinant DNA; A Short Course," 96-97, 192-193 (W.H. Freeman & Co., New York, 1983) (APPENDIX 6), describe the use of leader sequences at the NH₂-terminal ends of secretory proteins.

Because the claims, read in light of the specification, clearly define the claimed intact and essentially homogeneous hPTH(1-84) protein, the claims are definite. Accordingly, it is respectfully requested that the Board reverse the Examiner's rejection of claims 33-35 under § 112, second paragraph.

**II. CLAIMS 31 AND 32 ARE PATENTABLE UNDER
35 U.S.C. §§ 102(b) AND/OR 103 OVER BREWER ET AL.**

The Examiner rejected claims 31 and 32 under 35 U.S.C. § 102(b) as being allegedly anticipated by or, in the alternative, under 35 U.S.C. § 103 as being allegedly obvious over Brewer et al. (U.S. Patent No. 3,886,132) (APPENDIX 7). Office Action at pages 3-4. Applicants respectfully traverse this ground for rejection.

Brewer et al. purportedly relates to the isolation of hPTH from deceased human dried, defatted, parathyroid adenomas. This reference does not teach an intact essentially homogeneous hPTH(1-84) protein. Maggio Decl. at ¶ 9.

**A. Brewer et al. do not Provide any Purity or
Biological Data for an Intact hPTH Protein**

Brewer et al. do not provide any results to support the claim that the allegedly purified human parathyroid hormone migrated as a single component on an electrophoresis gel. Col. 2, lines 50-53, of Brewer et al. Radioimmunoassay techniques were allegedly used to isolate the protein, but no results from such tests are provided. Col. 2, lines 20-22, of Brewer et al. Further, no biological activity analysis of the intact hormone is provided. The only biological data provided was for a chemically synthesized hPTH sequence. Col. 8, lines 48-53; and col. 12, lines 9-13, of Brewer et al.

Moreover, the Maggio Declaration teaches that the combination of gel filtration and ion exchange chromatography did not obtain an essentially pure, or substantially homogeneous, hPTH: "[T]wo later publications cited by [the examiner], namely Kimura et al. and Kumagaye et al. show that the purification protocols discussed in Brewer et al. result in impure materials." Maggio Decl. at ¶ 9. Copies of Kimura et al. and Kumagaye et al. are attached as APPENDICES 8 and 9.

Furthermore, the intent of Brewer et al. was *Says that sequencing error proves that prtn was not intact* **not** to obtain a highly purified intact hPTH protein. Rather, the intent of Brewer et al was to obtain sufficient hPTH material to allow for sequencing of the N-terminal region of hPTH. Col. 1, lines 62-65, of Brewer et al. Homogeneity is not needed for sequencing. However, Brewer et al. failed to meet their own goal because they were unable to purify their hPTH sample sufficiently to allow for

correct sequencing. The lack of purity of the hPTH material of Brewer et al. is evidenced by Fig. 3 of Brewer et al., "Human Parathyroid Hormone: Amino Acid Sequence of the Amino Terminal Residues 1-34," *PNAS, USA*, 69:3585-3588 (1972) (APPENDIX 10) (this publication describes the invention disclosed and claimed by Brewer et al.) Fig. 3 illustrates the sequencing cycles of the N-terminal region of hPTH. In Fig. 3, several of the sequencing cycles illustrate more than one significant peak. The most likely explanation for the presence of such peaks is the presence of "sequenceable impurities." In addition, as the relative peak heights of these additional peaks often approaches that of the peaks attributed by Brewer's group to the actual amino acids in the sequence, the relative concentration of the sequenceable impurities rivals the concentration of hPTH in the sample. The ambiguity that these levels of sequenceable contaminants caused is the significant factor in Brewer et al.'s inability to properly identify the amino acids in positions 22, 28, and 30.

**B. Brewer et al. Only Characterize the
N-Amino Terminal Region of hPTH**

The intent of Brewer et al. was *not* to identify, isolate, and characterize an intact hPTH. Rather, Brewer et al. focused on the N-terminal region of hPTH. This is because Brewer et al. incorrectly teach that the "biological activity of the human [PTH] hormone . . . lie[s] in the first 34 residues." Col. 1, lines 56-59, of Brewer et al.

Human PTH is a hormone with multiple actions in different organ systems, such as bone and kidney, and these effects are conveyed through two different receptors. One of the receptors recognizes the N-terminal (first 30 amino acids) and the other receptor recognizes only the C-terminal part of the molecule, where full binding occurs between amino acids 19-84 and partial binding at amino acids 39-84. This means that only one amino acid substitution may change the hormone so that its receptor type binding and actions may differ dramatically from the protein having the correct sequence. Modifications in the N-terminal region could produce agonists with possibly increased affinity for PTH receptors or antagonists that bind to the receptor but which are biologically inactive. *irrelevant.*

Until recently, it was assumed that C-terminal fragments of PTH were biologically inert. *See e.g.*, col. 1, lines 48-58, of Brewer et al. However, new evidence

implicates an essential role for the C-terminus in the biosynthetic processing and secretion of the hormone by the parathyroid gland. See Inomata et al., "Characterization of a Novel Parathyroid Hormone (PTH) Receptor with Specificity for the Carboxy-Terminal Region of PTH(1-84)," *Endocrinology*, 136:4732-4740 (1995) (APPENDIX 16). Therefore, the biological actions of N-terminal or C-terminal fragments cannot separately mimic all of the biological effects of intact hPTH.

**C. The N-Terminal Amino Acid Sequence
of hPTH of Brewer et al. is Incorrect**

Brewer et al. describe the 34 amino acid N-terminal region of hPTH. However, Fig. 1 of Brewer et al. contains three errors in the N-terminal region of hPTH at positions 22, 28, and 30. Maggio Decl. at ¶ 9. Thus, it is not clear from the teaching of Brewer et al. whether the reference teaches isolation of an intact hPTH. Maggio Decl. at ¶ 9. This is significant because contrary to the teaching of Brewer et al., the biological activity of hPTH is *not* found solely in the N-terminal region. A mistake in the N-terminal region of hPTH could change the activity of the C-terminal region as it could alter the reading frame or a binding epitope of hPTH.

For at least these reasons, it is respectfully requested that Brewer et al. do not teach the claimed invention and, therefore, the Board should reverse the Examiner's rejection of the claims.

**II. CLAIMS 31-34 ARE PATENTABLE UNDER 35 U.S.C. § 103 OVER
BREYEL ET AL., SUNG ET AL., MAYER ET AL., AND KAISHA ET AL.**

Claims 31-34 were rejected under 35 U.S.C. § 103 as being allegedly unpatentable over Breyel et al. ("Synthesis of Mature Human Parathyroid Hormone in *Escherichia coli*," 3rd Eur. Cong. Biotechnol., 3:363-369 (1984)) or Sung et al. ("Hybrid Gene Synthesis: Its Application to the Assembly of DNA Sequences Encoding the Human Parathyroid Hormones and Analogues," *Biochem. Cell. Biol.*, 64:133-138 (1986)) or Mayer et al. (EP 0 139 076) or any reference of the three in view of Kaisha et al. (GB 2 092 596). Applicants respectfully traverse this ground for rejection.

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A. Breyel et al.

Breyel et al. (APPENDIX 11) is directed to expression of mature parathyroid hormone (1-84) in *E. coli* under control of a lac-promoter and a trp-promoter. See page 363, "Summary," of Breyel et al. This reference is discussed by Applicants in their specification, where it is noted that Breyel et al. demonstrated that *E. coli* degrades human PTH. See page 2, line 34, through page 3, line 8, of the Application. In particular, Breyel et al. teaches that "the half-life of PTH at the log phase was 30 min., . . . at stationary phase 15 min." See page 366 of Breyel et al. Thus, from the moment the hPTH is produced by *E. coli* in the method of Breyel et al., the hPTH composition will be contaminated by PTH fragments produced by the degradation of PTH. The degradation and subsequent inactivation of intact PTH is caused by internal protease sensitive domains in PTH. See Mathavan et al., "High Level Production of Human Parathyroid Hormone in *Bombyx mori* Larvae and BmN Cells Using Recombinant Baculovirus," *Gene*, 167:33-39, at 34 (1995) (APPENDIX 12). Accordingly, Breyel et al. **do not** teach an intact and substantially homogeneous hPTH protein, as claimed by Applicants.

B. Sung et al.

Sung et al. (APPENDIX 13) describes chemically synthesizing DNA sequences coding for the short fragments PTH(1-34) and PTH(1-40), and then using these sequences to construct a plasmid having the sequence for hPTH(1-84). See Sung et al. at 137-138. However, Sung et al. **did not** produce recombinant hPTH protein. In fact, this reference teaches that "[s]tudy is now conducted in the expression of these gene products." See Sung et al. at 138. A plan to conduct **future** research **does not** teach or suggest Applicants' claimed invention. *In re Deuel*, 34 U.S.P.Q.2d 1210 (Fed. Cir. 1995), citing *In re O'Farrell*, 7 U.S.P.Q.2d 1673, 1680-81 (Fed. Cir. 1988).

Moreover, had Sung et al. inserted the plasmids described in the reference in an *E. coli* expression system, intact and essentially homogeneous hPTH(1-84) would not have been produced, as prior to the present invention, such an expression system resulted in hPTH

compositions containing hPTH degradation products. *See e.g.*, Breyel et al. Accordingly, Sung et al. do not teach or suggest Applicants' claimed invention.

C. Mayer et al.

Mayer et al. (APPENDIX 14; and an English-language Abstract of the German-language document) refers to the production of hPTH in procaryotic and eucaryotic cells (but not yeast) using a gene isolated from a human genomic library using a probe made from pig cDNA. The exon nucleotide sequences for hPTH are shown in Fig. 2.

For experiments with *E. coli*, Mayer et al. allegedly transformed *E. coli* with expression vectors having the general outline given at page 16, no. 11. After growing the cells to middle log phase, the bacteria were centrifuged, treated with guanidine hydrochloride, and sonicated. This mixture was centrifuged and the supernatant allegedly contained PTH. The protein was precipitated and solubilized after isolation in HCl. This crude extract allegedly contained hPTH as demonstrated immunologically using antiserum against amino acid sequences 1-34, 28-48, and 48-68 in a radioimmunoassay.

No data regarding the results of the radioimmunoassay tests is given. Moreover, no data regarding the purity of the alleged hPTH protein is given. No data is given regarding the protein weight of the immunoreactive hPTH. No data is given regarding the amino acid sequence or composition of the protein produced in the *E. coli* expression system. Nor do Mayer et al. demonstrate that an intact hPTH(1-84) protein is produced. Given the teaching of Breyel et al., one of skill in the art would conclude that the protein produced by Mayer et al. in an *E. coli* expression system would be contaminated with degradation products of hPTH.

For experiments with eucaryotic cells, monkey kidney cells were transformed by means of the calcium phosphate method. The kidney cells were tested for the presence of PTH through extraction and radioimmunoassay. Thereafter, the hPTH gene (lambda-human DNA) was transformed into T3 cells together with the thymidine kinase gene (TK-gene) from Herpes simplex. TK positive clones were tested for the presence of the hPTH gene using

DNA hybridization (Scheme 1, page 10, of Mayer et al., shows the cDNA and amino acid sequences of prepro PTH, which is a 110 amino acid precursor to hPTH(1-84)).

No data regarding the results of the hybridization experiments are shown. Moreover, no data regarding the purity of the alleged hPTH protein is given. No data is given regarding the protein weight of the hPTH produced. No data is given regarding the amino acid sequence or composition of the protein produced in the *E. coli* expression system. Nor do Mayer et al. demonstrate that an intact hPTH(1-84) protein is produced.

In sum, Mayer et al. do not provide any data to substantiate the claims of producing recombinant hPTH in *E. coli* and mammalian kidney cells. As described by Breyel et al., Mathavan et al., and in Applicants' specification, *E. coli* expression systems were known to produce impure hPTH compositions contaminated with degradation products of hPTH. Mayer et al. do not teach intact, substantially homogeneous hPTH(1-84) protein, nor does this reference teach or suggest how to obtain such a protein. Accordingly, Mayer et al. do not teach or suggest Applicants' claimed invention.

D. Kaisha et al.

Kaisha et al. (APPENDIX 15) is directed to a process for producing hPTH in human lymphoblastoid cells comprising transplanting the cells to a non-human warm-blooded animal or by culturing the human cells. See col. 1, page 1, of Kaisha et al. The reference teaches that "[s]ince the use of such human lymphoblastoid cells results in the formation of easily disaggregatable massive tumors when the cells are transplanted to the animal body, and the massive tumors are barely contaminated with the host animal cells, the multiplied live human lymphoblastoid cells can be harvested easily." See col. 2, lines 2-73, at page 1 of Kaisha et al. Continuing, the reference teaches that the "hPTH thus obtained can be collected easily by purification and separation techniques using conventional procedures . . ." See col. 1, line 55, through col. 2, line 1, of page 2 of Kaisha et al.

This reference does not teach an intact and essentially homogeneous hPTH(1-84) protein. No data is provided regarding characterization of the alleged hPTH protein produced, including no molecular weight data, no gel electrophoresis confirmation data, and

no amino acid sequence or amino acid composition data. In sum, there is no data whatsoever indicating that Kaisha et al. teach or suggest an intact hPTH(1-84) protein.

Moreover, Kaisha et al. do not teach an intact *essentially homogeneous* hPTH (1-84) protein. The culturing method of Kaisha et al. can produce analogs of hPTH, inactive forms of hPTH, and hPTH fragments. Kaisha et al. do not teach or suggest how to remove such contaminants from the hPTH culture medium. Accordingly, Kaisha et al. do not teach or suggest the claimed invention, either alone or in combination with Breyel et al., Sung et al., or Mayer et al.

* * * *


Because the cited references do not teach or suggest, either alone or in combination, an intact substantially homogeneous hPTH(1-84 protein), it is courteously requested that the Board reverse the Examiner's rejections of the claims.

IV. CONCLUSION

The Board is respectfully requested to reconsider and reverse the outstanding rejections.

Respectfully submitted,

May 26, 1999
Date


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